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Effects of bestatin on phagocytic cells in cyclophosphamide-treated mice

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Abstract:
The low-molecular weight dipeptide bestatin is a potent inhibitor of aminopeptidase N and has been demonstrated to have antitumor and immunomodulatory effects. The effects of bestatin on interleukin (IL)-1β synthesis and release by peritoneal macrophages stimulated *in vitro* with lipopolysaccharide (LPS) from *E. coli,* the phagocytic and oxidative burst activity from peripheral blood monocytes and granulocytes and the number of blood leukocytes and blood picture in cyclophosphamide-treated mice were tested. Bestatin at doses of 1 and 0.1 mg/kg was injected into cyclophosphamide-treated mice *ip* five times on alternating days or ten times at 24 h intervals. The first dose of bestatin was administered 24 h after a single injection of cyclophosphamide at a dose of 350 mg/kg. It was found that bestatin administered at doses of 1 and 0.1 mg/kg five times on alternating days increased the synthesis and release of IL-1β by resident peritoneal murine macrophages stimulated *in vitro* with LPS in cyclophosphamide-treated mice. The immunocorrecting action of bestatin on the picture of peripheral blood in cyclophosphamide-treated mice was primarily observed with young forms of neutrophilic granulocytes. The changes were observed irrespective of the dosage and the number of subsequent doses applied. Moreover, the administration of bestatin after pharmacological immunosuppression partially prevented the suppressive effects of cyclophosphamide on the oxidative burst activity of peripheral blood monocytes and stimulated the phagocytic activity of granulocytes.

Key words:
bestatin, cyclophosphamide, phagocytosis, oxidative burst, IL-1β, mice

Abbreviations: APN – aminopeptidase N, BE – bestatin, CD – cluster of differentiation, CFU-GM – colony forming unit-granulocyte/macrophage, Con A – concanavalin A, CSF – colony stimulating factor, CY – cyclophosphamide, DCH – delayed-cutaneous hypersensitivity, DTH – delayed-type hypersensitivity, FBS – fetal bovine serum, FMLP – formyl-methionyl-leucyl-phenylalanine, GM-CSF – granulocyte/macrophage colony stimulating factor, HLA-DR – major histocompatibility complex 2, IL – interleukin, LPS – lipopolysaccharide, LTBMC – long-term human bone marrow cultures, MIP-1α – macrophage inflammatory protein, PBS – phosphate buffered saline, PMA – phorbol 12-myristate 13-acetate, TGF-β – transforming growth factor

Introduction

Macrophages, monocytes and neutrophils are phagocytes that are important in the immunological response and immunity of macroorganisms. The cells primarily function in innate immunity because they are not able to recognize the antigens specifically; however, they are also involved in the mechanisms of acquired immunity. Therefore, the ability to modulate the functions of these cells may be therapeutically beneficial for the treatment of many infectious diseases.

Bestatin (ubenimex), a low-molecular weight dipeptide, is known to be a biological response modifier [21]. The drug is also known for its antitumor [7, 10, 11, 22, 24], antibacterial [9], antiviral [4, 30, 31] and antifungal [2] effects as a result of a direct activity in the cells of the immune system. Moreover, this agent stimulates the activity of macrophages [37]. As shown previously, bestatin activates the tumoricidal properties in mouse peritoneal macrophages *in vitro* and tumor cytotoxicity in macrophages of mice treated with the drug [33]. Bestatin also increases the cytotoxic activity of peripheral blood lymphocytes and spleen cells of cancer patients due to the activation of macrophages [15]. In addition, the mitogenic action of this immunomodulator on lymphocytes is also connected with the stimulation of macrophages [14].

Previous studies have shown that bestatin has many diverse effects on the production of cytokines [19, 25, 27, 38]. It stimulates the humoral immune response [17, 31, 39], hematopoiesis [1], augments delayed-type hypersensitivity (DTH) to SRBC and restores the impaired DTH to SRBC and delayed cutaneous hypersensitivity (DCH) to oxazolone [13, 39]. Bestatin inhibits aminopeptidases, especially aminopeptidase N (APN) [3, 18, 36], which is identical to the cell surface molecule CD13 [20]. Membrane-bound APN/CD13 is distributed in hematopoietic cells of myeloid origin and outside of the hematopoietic system in epithelial, endothelial and fibroblast cells. Overexpression of APN/CD13 has been observed in various inflammatory diseases and cancers [3]. As an inhibitor of APN/CD13, bestatin has shown beneficial effects in the treatment of some inflammatory diseases and types of cancer [3, 28, 29]. It is currently used in Japan as an immunomodulator and antitumor drug.

However, there are little data about the influence of bestatin on the immune response impaired by immunosuppressants. Therefore, the purpose of the present study was to determine the effects of bestatin in different dosages and schedules of treatment on the activity of peritoneal macrophages and peripheral blood monocytes and granulocytes as well as on the number of blood leukocytes and blood picture in cyclophosphamide-treated mice. Cyclophosphamide, an alkylating agent used in cancer treatment and autoimmune diseases, is also used in experimental immunopharmacology to induce immunosuppression and estimate the immunocorrecting action of the drugs or substances considered as immunomodulators.

Materials and Methods

Animals

The studies were conducted on female Balb/c mice (8 weeks of age), with each weighing 20 g. The mice were kept under conventional conditions. The animals were fed a commercial, granulated food and water *ad libitum*. The experimental animals were obtained from the Breeding Center of Laboratory Animals at the Institute of Occupational Medicine, Łódź, Poland. The principles of laboratory animal care (NIH publication No. 86–23, revised 1985) as well as the specific national laws on the protection of animals were followed. The study protocol was approved by the Local Ethics Committee in Wrocław, Poland (No. 08/2008).

Drugs and treatment

Pharmacological immunosuppression was induced by a single intraperitoneal (*ip*) injection of cyclophosphamide (Endoxan – Baxter) administered at a dose of 350 mg/kg. Bestatin (in subst., Sigma) at doses of 1 and 0.1 mg/kg was injected into cyclophosphamide-immunosuppressed mice *ip* five times at 48-h intervals or ten times at 24-h intervals. The first dose of bestatin was administered 24 h after the cyclophosphamide injection. Bestatin and cyclophosphamide were dissolved in phosphate buffered saline (PBS, Institute of Immunology and Experimental Therapy, Wrocław, Poland). The trials in the control groups were conducted in parallel. The mice in the control groups received PBS instead of bestatin or cyclophosphamide. The volume of each dose of bestatin, cyclophosphamide or PBS was 0.2 ml per animal. Each experimental group consisted of eight mice.

Measurements

The following indices were measured: (i) the production of interleukin-1β (IL-1β) in the culture supernatants of peritoneal macrophages stimulated *in vitro* with lipopolysaccharide (LPS) from *E. coli* (055:B5, Sigma); (ii) the number of leukocytes in the blood and blood picture; (iii) the phagocytic activity of the blood monocytes and granulocytes; and (iv) the oxidative burst activity of the blood monocytes and granulocytes. The level of IL-1β was determined 24 h after